**T-cells and molecular prognosis profiling in breast cancer**

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**INTRODUCTION:** Molecular prognosis profiles are better at predicting outcome in breast cancer than nodal status. Adequate immune-surveillance for cancer cells and the anti-tumour function of these cells is essential for survival in breast cancer patients. Here we examine the systemic T-cell functional profile in patients with breast disease.

**METHODS:** Sixty patients undergoing surgical treatment for breast cancer or benign breast disease were prospectively recruited and preoperative blood samples were obtained. Mononuclear cells were separated using density gradient technique and activated using the T-cell mitogen staphylococcal enterotoxin B (SEB). Following culture interleukin 2 (IL2) ELISA was performed to assess T-cell function and patients were followed.

**RESULTS:** Malignancy was confirmed histologically and systemic disease staged radiologically. Multivariate patient characteristic analysis revealed no differences between patient groups. IL2 levels were significantly depressed in cancer patient T-cells (2817.9 vs. 1692.1, p < 0.05 (benign vs. malignant) and 1304.8 vs. 1965.4, p < 0.05 (node positive vs. node negative cancer) pg/ml). At mean follow up of 18.7 months those patients with relapse produced less T-cell IL2 (400.9 vs. 1953.3, relapse vs. non relapse, pg/ml).

**CONCLUSIONS:** Breast cancer patients demonstrate a depressed T-cell IL2 response, which appears to be not only disease specific but also stage specific. Breast cancer patients who relapse have much lower preoperative T-cell IL2 production than non-relapers. These results suggest that T-cell IL2 production could be used as part of a molecular prognosis profile in breast cancer patients.

**Gene expression signature characterizing cyclin E protein overexpression in primary breast tumors**

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**INTRODUCTION:** Cyclin E protein overexpression has been demonstrated to correlate with recurrence in breast cancer, and does not correlate with gene expression on Affymetrix Hu95 microarrays. We sought to identify a gene expression signature characterizing cyclin E protein overexpression using gene expression data on primary breast tumors.

**METHODS:** RNA and protein were extracted from forty-four breast carcinoma specimens from the Duke University Breast Cancer SPORE frozen tissue bank. Fluorescently labeled cDNA probe was generated and hybridized to Affymetrix Hu95 gene chips in the Duke University Microarray Core Facility yielding gene expression data. Cyclin E protein expression was measured by tissue western blot. Prediction Analysis of Microarrays software and clustering algorithms were used to identify differentially expressed genes and to make cyclin E protein overexpression predictions.

**RESULTS:** Of 44 tumors, 12 exhibited cyclin E protein overexpression. There was no correlation between protein overexpression and cyclin E gene expression on the gene array. The 91 genes

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**Lipoxygenase expression in colon polyps and inhibition of colon cancer growth by lipoxygenase blockade**

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**INTRODUCTION:** Expression of 5-lipoxygenase (5-LOX) has been shown to be up-regulated in early pancreatic cancer, but has not been characterized in early colon neoplasms. We hypothesize that there is upregulation of 5-LOX in colon polyps and that inhibition of 5-LOX in colon cancer cell lines would inhibit cell growth and induce apoptosis.

**METHODS:** Tissue samples of normal colonic mucosa and neoplastic polyps were collected from 20 patients. Immunohistochemistry was used to compare 5-LOX expression between patient’s normal colonic mucosa and polyps. Effects of the specific 5-LOX inhibitor, Rev 5901, on cell proliferation were studied in colon cancer cell lines (LoVo, HT-29, HCT-116).

**RESULTS:** Intense 5-LOX staining was evident in polyps from 20/20 specimens. Adjacent areas of normal colonic mucosa did not express 5-LOX. Specific 5-LOX blockade with Rev 5901 showed concentration and time-dependent inhibition of cell proliferation (85% reduction relative to control with 15uM at 24 hours, p < 0.005) of all three cell lines. TUNEL assay revealed that Rev 5901 induced apoptosis in colon cancer cells (12%-treated vs. 0.9%-control, p < 0.01) which was confirmed by DNA fragmentation assay and annexin V binding.

**CONCLUSIONS:** These results demonstrate that 1) 5-LOX is up-regulated in colon polyps compared to normal mucosa, and 2) treatment with 5-LOX inhibitor Rev 5901 results in decreased cell proliferation and increased apoptosis. These findings provide evidence that 5-LOX plays a role in colon cancer development and may be an early target for chemoprevention of colon cancer.
Expression of interferon receptors in pancreatic cancer: Identification of a novel prognostic factor
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INTRODUCTION: Interferons (IFN) are known to have antiproliferative and immunoregulatory activities that are modulated through IFN receptors.

METHODS: Expression of IFN-alpha/beta receptor-chain 2 (IFNalpha/betaR) and IFN-gamma receptor (IFNgammaR)-chain 1 was examined in 46 patients with pancreatic adenocarcinoma using immunohistochemistry (IHC).

RESULTS: The IHC performed for pancreatic adenocarcinoma demonstrated a high IFNalpha/betaR expression in 4.3% (2/46) of patients, moderate expression in 19.6% (9/46) of patients, and faint or no expression in 76.1% (35/46) of patients. IHC confirmed a high expression of IFNgammaR in 52.2% (24/46) of patients, moderate expression in 34.8% (16/46) of patients, and faint or no expression in the remaining 13% (6/46) of patients. By comparison, 28.2% (13/46) and 45.6% (21/46) of the corresponding non-cancerous pancreatic tissue showed a high expression of IFNalpha/betaR and IFNgammaR, respectively. Clinico-pathological survey did not demonstrate a significant correlation between IFNalpha/betaR and IFNgammaR expression with regard to tumor size, vascular invasion, perineural invasion, lymph node metastasis, or stage of disease. However, Kaplan-Meier analysis demonstrated a significant survival advantage in those patients whose tumors expressed moderate-high IFNalpha/betaR expression compared to those with faint or no IFNalpha/betaR expression (22 months vs. 13 months; p = 0.012, log rank test). The expression of IFNgammaR, however, had no impact on patient survival (20 months vs. 17 months; p = 0.656, log rank test).

CONCLUSIONS: IFNalpha/betaR is an independent prognostic factor in pancreatic cancer and is a potential candidate for biologic treatment of pancreatic cancer.

The ubiquitin ligase subunits Skp2 and Cks1 are novel independent prognostic markers for survival in colorectal cancer
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INTRODUCTION: Loss of the cell-cycle inhibitory protein p27 is associated with aggressive tumor behavior and poor prognosis in colorectal cancer. The decrease in p27 levels is the result of increased proteasome-dependent degradation, mediated and rate-limited by its specific ubiquitin ligase subunits Skp2 and Cks1. We recently found that overexpression of Skp2 and Cks1 in colorectal cancer correlated with low p27 levels and poor tumor differentiation. The potential role of Skp2 and Cks1 as independent prognostic markers, however, is unknown.

METHODS: Tissue samples from 100 patients operated for colorectal cancer at 1997 were subjected to Western blot analysis and immunohistochemistry using highly specific monoclonal antibodies against p27, Skp2 and Cks1. Results were plotted against patients’ characteristics, disease stage and overall survival using Cox analysis and the Kaplan-Meier method.

RESULTS: Skp2 and Cks1 expression strongly correlated with overall survival (HR 7.672; p < 0.001 and HR 5.026; p < 0.001, respectively). Thus, high levels of these proteins, alone or in combination, accurately predicted poor prognosis whereas low levels predicted good overall survival rates. The strength of these proteins as independent prognostic markers was also valid after controlling for age, sex, grade and stage. Moreover, after having stratified for p27 levels, both Skp2 and Cks1 expression significantly enhanced the predictive value for survival (HR 4.530; p < 0.001 and HR 4.236; p < 0.001, respectively). The strongest additive effect was observed in patients with stage II disease.

CONCLUSIONS: Skp2 and Cks1 expression strongly correlate with overall survival and may thus be used as novel prognostic markers in colorectal cancer.

Flavopiridol inhibits soft tissue sarcoma growth with preferential sensitivity in a Cdk4 gene amplified subtype
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INTRODUCTION: Current chemotherapy for advanced soft tissue sarcoma (STS) has low overall response rates. It has been shown that the CDK4 gene is frequently amplified in some STS subtypes. Here we tested flavopiridol, a pan cyclin dependent kinase inhibitor, with STS subtypes that have amplified (dedifferentiated liposarcoma,